

EVALUATING GROWTH CHARACTERISTICS AND TOTAL ANTHOCYANIN CONTENT IN THREE CULTIVARS OF RED ROMAINE-TYPE LETTUCE (*Lactuca sativa* L.) IN RESPONSE TO THREE LIGHTING INTENSITIES

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ABSTRACT

The ability to provide fresh food in the diet of a crew on long duration space missions can have significant physiological and psychological benefits. Providing a sufficient quantity and variety of fresh crops as well as providing colorful foods may further increase the benefit. Due to the power limitations and air filtration systems in a spacecraft, space-grown plants will likely be grown in an open systems sharing the same atmosphere as the crew, consisting of high CO₂ concentrations and low lighting levels. In this test, three cultivars of red romaine-type lettuce, “Outredgeous”, “Eruption”, and “Red Sails”, were grown hydroponically under three different light intensities; 8.6, 17.2, and 26 mol m⁻² d⁻¹ to determine the yield characteristics. In addition, as a potential criteria for selection of a cultivar, total anthocyanin content (i.e. red coloration) of each cultivar was determined. Of the three cultivars, the one with the optimal growth characteristics and anthocyanin content under the spaceflight environmental conditions will be selected as a possible candidate crop to be grown on the International Space Station (ISS), a Mars transit vehicle, or Martian and lunar bases. Results indicated that “Red Sails” had the highest edible fresh weight at all light intensities, but its stature may not be conducive for the limited volume of a plant growing unit or as part of mixed crop production system. Both “Eruption” and “Outredgeous” had similar anthocyanin contents among all light intensities and were nearly three-fold higher than “Red Sails”. However, “Outredgeous” had greater yield than “Eruption” and therefore may be the best suited cultivar for the use in a space application.

INTRODUCTION

NASA’s Advanced Life Support program is currently testing crop plants for inclusion in bioregenerative life support systems for long-duration missions or for dietary augmentation for near-term missions. Plants such as lettuce, onions, radishes, tomatoes, peppers, and strawberries are currently being grown under baseline ISS environmental conditions to determine how they will perform under environmental conditions typical of an open cabin atmosphere (1, 2). Providing fresh food for the crew of ISS or future Mars missions could have physiological and potential psychological effects, including direct nutritional benefits, nutraceutical benefits, and the visual impact of different colors inside the spacecraft (1). Environmental conditions are limited on a spacecraft, as there exists such factors as high CO₂ levels (>1200 μmol mol⁻¹) and low lighting intensities. However, previous studies have shown that salad crops can perform well under the anticipated ISS baseline environmental conditions (2).

Although green lettuce is a typical salad crop, red lettuce contains anthocyanins, which are responsible for the red pigmentation, and are potent antioxidants. Crew diets containing anthocyanins could provide a countermeasure to the effects of cosmic radiation. In this study, three cultivars were evaluated for their growth characteristics and anthocyanin content under three lighting intensities that are currently being studied as part of the ISS baseline environmental conditions.

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MATERIALS AND METHODS

Three cultivars of lettuce (*Lactuca sativa* L. cvs. “Eruption”, “Outredgeous”, and “Red Sails”) were grown in a 48 ft² walk-in controlled environment chamber at Kennedy Space Center’s Space Life Sciences Lab Phytotron under the following environmental conditions and experimental parameters:

- Air temperature 25°C
- Relative humidity 65%
- Fluorescent lighting
- 16-h light/8-h dark photoperiod
- Lighting intensities of 150, 300, and 450 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPF (8.6, 17.2, and 26 $\text{mol m}^{-2} \text{d}^{-1}$, respectively)
- Nutrient Film Technique hydroponics w/ ½ strength modified Hoagland’s solution
- pH 5.8
- Electrical conductivity 120 mS m^{-1}
- Carbon dioxide 1200 $\mu\text{mol mol}^{-1}$
- Harvest at 28 DAP
- Anthocyanin extraction w/ 1% acidified methanol measured at 530 nm: Standard used was cyaniding-3-glucoside

RESULTS

Table 1: Growth characteristics of the three lettuce cultivars at 28 DAP for each light intensity. Data represent means \pm S.E.; n=16.

| Cultivar | PPF | Leaf Number | Leaf Area (cm ²) | Dry Weight (g plant ⁻¹) | Dry Mass % | Diameter (mm) |
|-------------|------|----------------|------------------------------|-------------------------------------|--------------|-----------------|
| Outredgeous | 8.6 | 14.38 (0.69)a* | 854.98 (55.32)b | 1.50 (0.11)c | 4.72 (0.11)b | 309.22 (16.81)a |
| | 17.2 | 16.75 (0.54)a | 1194.17 (92.38)a | 2.40 (0.24)b | 4.00 (0.12)c | 317.81 (13.35)a |
| | 26 | 16.06 (1.28)a | 997.09 (52.79)ab | 3.39 (0.17)a | 5.48 (0.13)a | 280.94 (8.33)a |
| Eruption | 8.6 | 15.50 (0.58)b | 464.20 (22.70)b | 0.93 (0.06)c | 4.61 (0.14)b | 200.78 (6.55)a |
| | 17.2 | 17.88 (0.50)a | 691.94 (25.14)a | 1.50 (0.06)b | 4.11 (0.09)b | 214.06 (10.54)a |
| | 26 | 18.13 (0.70)a | 514.36 (28.23)b | 1.94 (0.09)a | 5.25 (0.30)a | 200.63 (23.43)a |
| Red Sails | 8.6 | 20.63 (1.23)a | 1655.71 (245.85)a | 2.19 (0.24)c | 4.15 (0.16)a | 328.44 (15.48)a |
| | 17.2 | 23.56 (1.50)a | 2095.94 (166.93)a | 4.16 (0.33)b | 3.91 (0.14)a | 281.88 (7.72)b |
| | 26 | 24.47 (1.95)a | 1943.62 (123.83)a | 5.08 (0.42)a | 4.13 (0.25)a | 319.27 (12.26)a |

*Means in columns, within cultivar, with the same letter are not significantly different according to the studentized T-test; p=0.05; n=16.

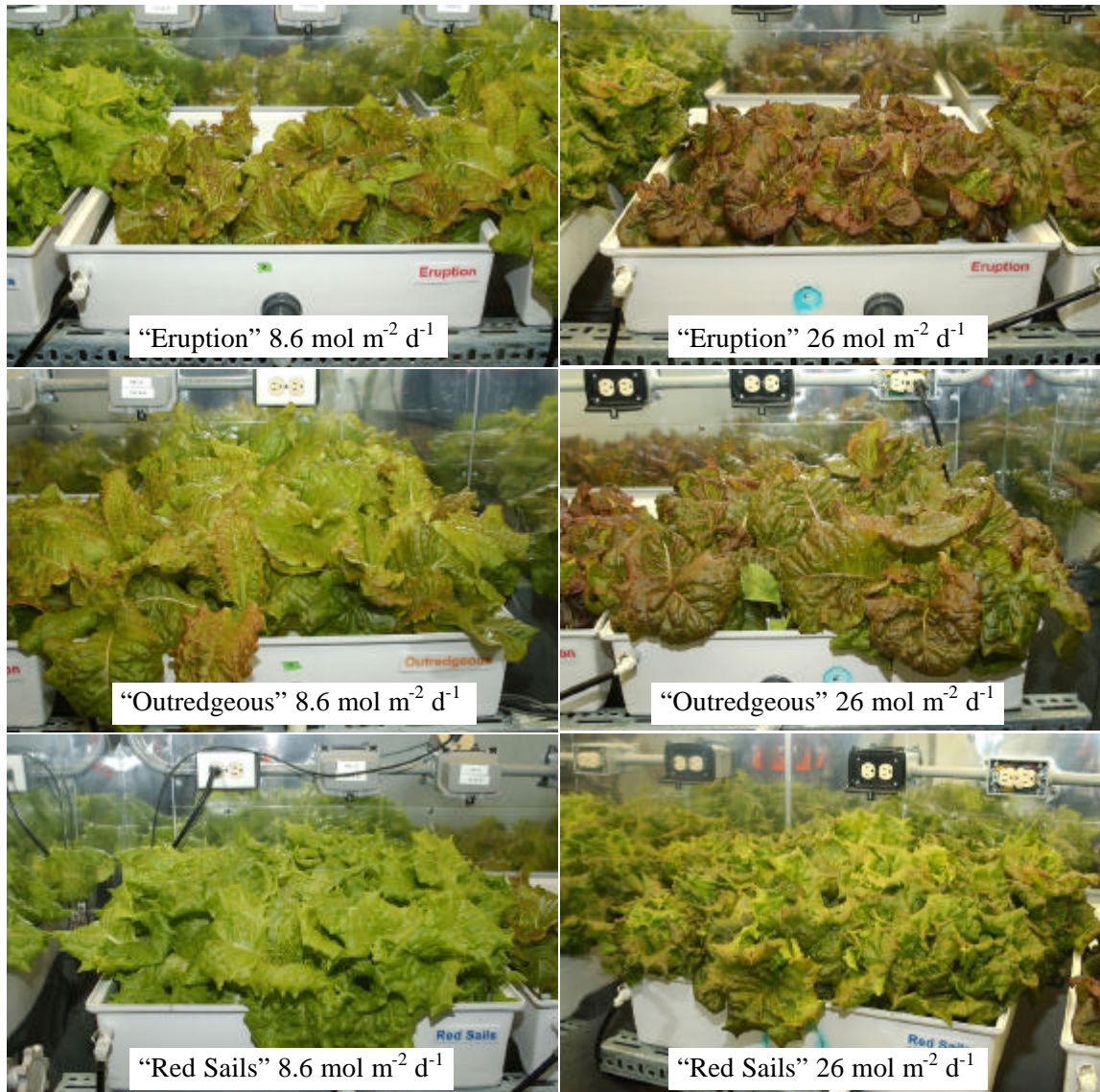


Figure 1. Lettuce plants at 28 days after planting (DAP) grown at 8.6 and 26 mol m⁻² d⁻¹. Note significant anthocyanin development at higher light.

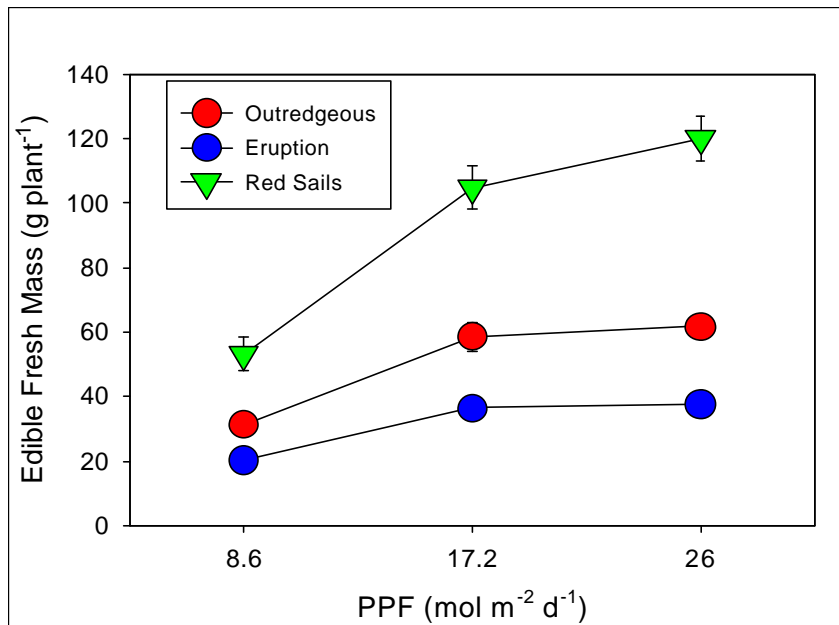


Figure 2. The mean edible fresh weight of cultivars Outredgeous, Eruption, and Red Sails grown at the three lighting intensities of 8.6, 17.2, and 26 mol m⁻² d⁻¹, at 28 DAP. Data represent means \pm S.E.; n=16.

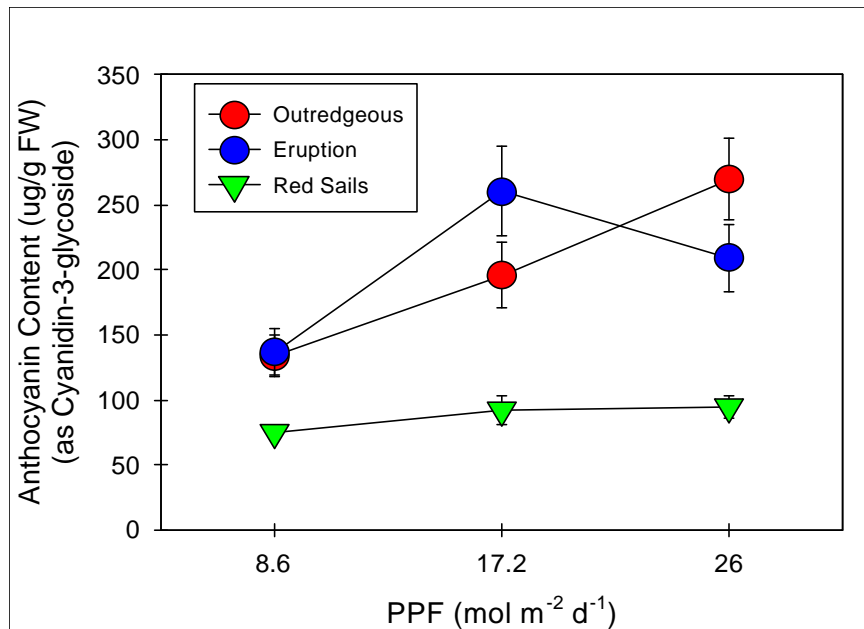


Figure 3: The mean total Anthocyanin content of cultivars Outredgeous, Eruption, and Red Sails grown at the three lighting intensities of 8.6, 17.2, and 26 mol m⁻² d⁻¹, at 28 DAP. Data represent means \pm S.E.; n=16.

DISCUSSION

In previous studies, lighting intensities has been shown to be a primary factor that affects plant growth in salad crops (2). Given that light intensities may be low on long duration space missions, these studies evaluated differences between light levels in terms of growth and anthocyanin production. Edible fresh weight was the primary growth characteristic evaluated in this cultivar selection as this represents what would be consumed by the crew. Increasing light intensity increased the edible fresh weight of all three cultivars (Figure 2). “Red Sails” had the highest edible fresh weight at 28 DAP. However, due to its large leaf morphology and non-compact growth habit (Table 1; Figure 1), it may be unsuitable due to the limited growing space available or in a mixed crop system as it may shade smaller, slower-growing crops.

Lighting intensity may be an important factor in the development of the red pigments when growing red romaine-type lettuce in an environmentally controlled environment, such as on the ISS. In general, total anthocyanin content increased in all cultivars as light intensity increased. Although “Red Sails” had the highest fresh weight, it had the lowest anthocyanin content per gram of fresh weight. “Outredgeous” and “Eruption” had a several-fold increase in anthocyanin content as compared to “Red Sails” and had a similar increasing trend over the increasing light intensities (Figure 3). As antioxidants are important in scavenging free radicals, this makes “Outredgeous” and “Eruption” the best cultivars because of their higher anthocyanin content. “Outredgeous” yielded better than “Eruption”, so it is therefore the cultivar suggested for further ISS baseline environmental studies.

CONCLUSION

- The cultivar with the highest fresh weight was Red Sails, but Outredgeous may be more preferable on the ISS due to space limitations.
- The cultivars with the highest anthocyanin content per gram of fresh weight were Eruption and Outredgeous with a 3-4 fold increase in content at the moderate $17.2 \text{ mol m}^{-2} \text{ d}^{-1}$ PPF, and high $26 \text{ mol m}^{-2} \text{ d}^{-1}$ PPF. Anthocyanin content continued to increase in Outredgeous as light levels increased.
- The above criteria show that Outredgeous is the optimal cultivar to continue in future ISS baseline environmental studies and possible lunar or Mars missions.

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